A STUDY OF ISOTONIC SOLUTIONS FOR THE ERYTHROCYTES OF SOME MARINE TELEOSTS AND ELASMOBRANCHS

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The assumption is commonly encountered that in all teleosts the osmotic pressure of the blood is equivalent to about 0.25 molar (M) NaCl and in all elasmobranchs to about 0.5 M NaCl (*cf.*, Baldwin, 1949; Davson, 1951; Prosser *et al.*, 1950; Heilbrunn, 1952). The evidence for these values was obtained by the determination of the bloods' freezing point depression, from which the osmotic pressure was calculated (Botazzi, 1897; Fredericq, 1901; Garrey, 1905; Rodier, 1899; Schlieper, 1930; Smith, 1932; Krogh, 1939). The number of species examined in each class was generally small and no special significance was attributed to the observed variations in blood osmotic pressure between different species.

In addition, few attempts have been made to develop solutions for fish tissues which were balanced with respect to both ion content and concentration. Those of Baglioni (1906) and Fry (1909) were based upon the urea analyses of elasmobranch blood by Schraeder (1890); that of Lutz (1930) upon the data of Smith (1929). The Baglioni and Lutz solutions were used in studies on the elasmobranch heart, while Fry's was used in a study of blood coagulation. The "Manual of Formulae and Methods (III)" of the Marine Biological Laboratory, Woods Hole, lists a few artificial solutions supposedly "physiological" for fish tissues. Our findings reported below indicate that these solutions are not isotonic for red cells of the species studied. Apparently no systematic studies have been undertaken to determine isotonic solutions for fish erythrocytes. Solutions calculated from ion analyses of body fluids, such as those of Smith (1929) cannot, without experimental study, be taken as isotonic for red cells.

We have found that a solution of NaCl which was isosmotic with a sample of fish serum, as determined by the depression of the freezing point, was hypertonic to the red cells of that fish. This general finding led us to investigate other methods for determining solutions which were isotonic for the red cells of teleost and elasmobranch bloods.

Materials and Methods

Blood was collected from the elasmobranchs by syringe from either the caudal vessels or the heart. In the teleosts blood was taken from the heart. In the early work, blood from mackerel and tautog was obtained from the severed branchial arteries. When a sample of blood from a single fish was less than 10 ml., blood from several fish of the same species was usually pooled for study. The blood was defibrinated by gentle stirring with a rubber-tipped glass rod. In a few cases sodium oxalate was used as an anticoagulent instead of defibrination. The blood was filtered through gauze to remove small clots and, if not immediately used, was

stored at 7° C. All measurements were made at about 23° C. with blood that was less than eight hours old. Blood was collected from all of the fish immediately after their removal from sea water. In most cases the fish were caught in a fish weir.

Four separate experimental approaches were used to investigate either cell volume changes or the osmotic concentration of the serum. These four were: (1) cryoscopy, (2) hematocrit, (3) hemoglobin concentration and (4) densimetry.

Cryoscopy, the only method which does not measure cell volume, was used to determine the osmotic concentration of serum and of equivalent NaCl solutions. This method is only useful in determining isosmotic solutions, not isotonic solutions.

Measurements of cell volume by hematocrit, hemoglobin concentration and densimetric methods were based on the assumption that cells are at osmotic equilibrium with their environment, *viz.*, serum. In addition these three methods depended upon the cells behaving as osmometers (*cf.* Lucke and McCutcheon, 1932). Confirmatory evidence for this idea is found in our experiment where the relative volume of fish red cells plotted against the reciprocal of the saline concentration of the suspension medium gave a linear relation. The concentration of NaCl in which the cells maintained the same volume as in serum was taken as isotonic.

TABLE I

Freezing point depressions of NaCl solutions

Solution of NaCl in moles per liter	ΔT_f
0.05	−0.197
0.10	−0.372
0.15	−0.576
0.20	−0.771
0.25	−0.943
0.30	−1.133
0.35	−1.328
0.40	−1.449
0.50	−1.816
0.75	−2.690
1.00	−3.692

The freezing point depression (ΔT_f) of serum or whole blood was measured with the standard Beckman apparatus by the method of Findlay (1941). The ΔT_f of a series of NaCl solutions of known molarity was also determined by the same method. The NaCl used in preparing these solutions was Merck reagent grade sodium chloride "for biological work" (Williams and Jacobs, 1931). From these data a calibration curve of ΔT_f versus known *molarities* of NaCl was prepared, and isosmotic solution of NaCl interpolated from this curve from the ΔT_f measurements of fish sera. Table I presents the data from which this curve was constructed. Our NaCl solutions were unbuffered and well-aerated.

The hematocrit method of Parpart and Ballentine (1943) was used to compare the volume occupied by a known quantity of red cells in a NaCl solution of known concentration. By the use of this method one was able to determine the particular NaCl solution in which the cells occupied the same volume as in serum.

Since the loss of hemoglobin from red cells is considered an all-or-none phenomenon (Parpart, 1931), the amount of hemoglobin in a given volume of cells

can be used as a measure of the quantity, *i.e.*, volume, of red cells present in a suspension (Parpart and Green, 1951). In practice, red cells in either serum or NaCl solutions were packed at 15,000 G for 10 minutes. Twenty cmm. of packed cells were hemolyzed in a given volume of 0.03 *M* NaCl containing 0.003 *M* phosphate buffer at pH 7.4. The concentration of hemoglobin was measured spectrophotometrically at 540 mμ. NaCl solutions which consistently maintained red cells at a volume, such that the hemoglobin concentration of packed samples was equal to the hemoglobin concentration of packed samples of cells in serum, were considered isotonic with serum.

The densimeter technique of Parpart (1935) was used to record rapid volume changes in red cells. This technique, which automatically records the amount of light transmitted through a red cell suspension, is capable of following volume changes of cells exposed to suspension medium from the first second onwards. By the use of the densimeter, NaCl solutions were found in which the volume of the cells was maintained constant and equal to that in serum. Since this instrument permitted the rapid determination of isotonic NaCl solutions, it was used chiefly in survey studies of a number of fish bloods.

TABLE II

Molar values of isotonic NaCl solutions
Species of fish

Method of determination	Mackerel			Tautog			Smooth dogfish					
	No. of fish	No. of dtm.	Value moles NaCl	No. of fish	No. of dtm.	Value moles NaCl	No. of fish	No. of dtm.	Value moles NaCl	No. of fish	No. of dtm.	Value moles NaCl-Urea†
Hematocrit	67	10	0.27±.007	21	7	0.21±.007	11	5	0.34±.008	14	7	0.47±.004
Hemoglobin	41	6	0.26±.005	6	3	ca. 0.23	10	4	0.35±.02	12	5	0.51±.009
Densimeter	67	10	0.26±.004	23	8	0.26±.007	13	6	ca. 0.53	14	7	0.52±.005
ΔT_f*	59	9	0.30±.004	23	8	0.27±.008	13	6	0.53±.003	13	6	0.53±.003

* Isosmotic solutions.

† Equal molecular amounts of NaCl and urea expressed as equivalent moles of NaCl.

RESULTS AND DISCUSSION

Table II presents values of isotonic solutions of NaCl for the red blood cells of mackerel, tautog and dogfish obtained by three of these methods. Also included in this table are isosmotic values for these cells as determined by ΔT_f measurements of their sera. The measure of variation is indicated by the standard error.

No significant differences in tonicity of NaCl solutions for a given species were obtained by the use of hematocrit and hemoglobin methods. Both of these methods measured the equilibrium volume attained by the cells after one hour of exposure to serum or NaCl solutions. Of the three species studied by these two methods, each would appear to require isotonic NaCl solutions of differing molarity. Statistically the differences between 0.27 *M* for mackerel, 0.21 *M* for tautog and 0.34 *M* NaCl for dogfish are real.

The densimeter measurements of isotonicity for these three species are not in

complete agreement with those obtained by hematocrit and hemoglobin methods. The most unsatisfactory measurement is that for tautog blood. We do not know why this value is high or, perhaps, why the hematocrit-hemoglobin values are low. If the hematocrit and hemoglobin values are low, the cells may have decreased their internal osmotic pressure. This is possible because these methods require a longer time to determine the isotonicity (between one and two hours) than does the densimeter method, which makes such measurements within a few minutes.

When dogfish red cells are taken from serum and placed in a NaCl solution which does not contain urea, urea diffuses from the cells and within nine minutes a new equilibrium volume is reached. But when the initial volume of dogfish red cells is obtained by use of the densimeter, by using only the first 10 seconds of exposure, it is found that a NaCl solution of 0.53 *M* is "isotonic." This is because no water exchange is possible now and the only volume change which will be observed will be shrinking as urea diffuses out of the cells. This volume change due to the outward diffusion of urea was not noticeable under our conditions of measurement until about 30 seconds after the cells were exposed to the 0.53 *M* NaCl solution. Thus the apparent discrepancy in isotonic volumes for dogfish red cells between densimeter and hematocrit measurements reflects the loss of urea in the latter. The urea plays no role in determining the final equilibrium volume of these cells as was shown by Green and Hoffman (1951).

The NaCl-urea solution indicated in Table II contained both urea and NaCl in 0.35 *M* concentrations. Such a mixture is equivalent osmotically to 0.53 *M* NaCl. Aqueous dilutions of this NaCl solution expressed as the equivalent moles of NaCl appear in Table II as isotonic solutions for dogfish red cells. The isotonic value of 0.47 *M* obtained by the hematocrit method is significantly different from that arrived at by other methods. This variance is not understood. The value of using NaCl-urea solutions is to prevent urea-containing cells from undergoing any volume change. The results shown in Table II indicate that the NaCl-urea solution, containing 0.35 *M* of each of these reagents, is approximately isotonic.

The column headed ΔT_f in Table II lists the isosmotic values in moles of NaCl of the sera of these three species of fish. In general these isosmotic values are higher than the isotonic values obtained by the other methods. In the case of mackerel serum this difference is statistically significant. The reason for this hypertonicity of isosmotic solutions may be attributed in part to the difference in the activity of ions at the freezing point and at room temperature. Of more importance is the response of the cell to a solution containing a single species of cation. In such a solution the cell surface may be modified or the rate of water penetration may be different from that in plasma. Either of these conditions could account for this hypertonicity.

Table III shows the actual freezing point depressions of the bloods of the fish in Table II. The percentage of cells of the teleosts was determined in defibrinated blood obtained from cutting the gills. These are somewhat lower than values found for blood obtained from heart puncture. The pH values are for aerated blood and quite similar to the pH of the blood of other vertebrates.

The data in Tables IV and V were obtained using the densimeter method for the determination of isotonicity. The accuracy of this method is $\pm$ 0.001 *M* of NaCl and the maximum variation (for blood from the heart or caudal vessels) among fish of the same species was $\pm$ 0.01 *M* NaCl.

TABLE III

Physical data on fish bloods

Kind of fish	No. sampled	No. of measurements	pH		% cells	Freezing point °C.
			Average	Range		
Mackerel	57	8	7.40	7.31–7.57	21.2±0.4*	−1.13
Tautog	17	5	7.27	7.08–7.48	20.5±0.8	−1.05
Dogfish	14	7	7.50	7.35–7.83	20.6±0.07	−1.95

* Standard error.

Table IV shows the dependence of the isotonic value upon the method of obtaining the sample of blood. The results with heart blood are thought to be closer to the true tonicity since the gill blood is probably contaminated with sea water and body fluids. Very striking is the fact that for either heart or gill blood there is a large variation in values between the various species of teleosts. The range is 0.10 *M* NaCl for the shark sucker to 0.26 *M* NaCl for the mackerel. These data necessitate a revision of the apparently over-generalized concept that the isotonic values for the teleosts fluctuate slightly around 0.25 *M* in NaCl, and also of our ideas concerning water balance in these forms.

Table V lists the isotonic values obtained for different elasmobranch bloods *after* osmotic equilibrium was established between the cells and the NaCl solution. In addition certain solutions listed in the "Formulae and Methods III" of the Marine

TABLE IV

Molar values of isotonic solutions for teleost red cells

Name of fish	No. studied	No. detn.	Average isotonicity Moles of NaCl	
			Blood drawn from	
			Gills	Heart
Shark sucker (*Echeneis naucrates*)	2	2	—	0.10
Weak fish (*Cynoscion regalis*)	1	1	—	0.13
Flounder (*Paralichthys dentatus*)	10	5*	0.16	0.14
Toadfish (*Opsanus tau*)	2	2	0.19	0.14
Eel (*Anguilla rostrata*)	1	1	—	0.15
Butter fish (*Poronotus tricanthus*)	4	2*	—	0.16
Sea robin (*Prionotus carolinus*)	5	4*	0.18	0.16
Menhaden (*Brevoortia tyrannus*)	19	8*	0.19	0.18
Sea bass (*Centropristus striatus*)	2	2	—	0.19
Porgy (*Stenotomus chrysops*)	8	3*	—	0.19
King mackerel (*Scombermorus cavalla*)	5	2*	0.21	0.19
Blue fish (*Pomatomus saltatrix*)	6	4*	0.25	0.20
Tautog (*Tautoga onitis*)	23	8*	0.27	0.21
Mackerel (*Scomber scombrus*)	65	10*	0.26	0.26

* Some measurements were made from pooled blood.

Table V

Molar values of isotonic solutions for elasmobranch red cells

Name of fish	Number studied	Isotonicity Molar NaCl	Elasmobranch soln.	Knowlton's soln.	Van't Hoff soln.	Lutz soln.
Smooth dogfish (*Mustelis canis*)	4	0.35	Hyper	Hypo	Hyper	Hypo
Sting ray (*Dasyatis centrura*)	2	0.33	Hyper	Hypo	Hyper	—
Skate (*Raja erinacea*)	1	0.34	Hyper	Hypo	Hyper	—
Sand shark (*Carcharias littoralis*)	1	0.35	—	—	—	—
			0.46*	0.41*	0.43*	

* Molar value of NaCl solutions of equivalent osmotic pressure as determined by ΔT_f measurements.

Biological Laboratory for use with elasmobranchs are evaluated. The value of the equivalence in moles of NaCl (obtained from the freezing point depression) of these solutions is also indicated. These data show that the range found in this group of fishes is from 0.33 *M* for the sting ray to 0.35 *M* NaCl for the sand shark and the dogfish. The range is very much less than that of the teleosts. The elasmobranch and Knowlton's solutions contain urea which explains the very large discrepancy between the evaluation of the isotonicity and the ΔT_f as discussed above.

Conclusions

The results require a closer appraisal of the physiological conditions for fish tissues suspended in artificial environments. This is particularly so for the teleosts since their bloods exhibit highly variable osmotic pressures. If the generally assumed value of 0.25 *M* NaCl were used, isotonicity would be approached only in the case of the mackerel. For the other teleosts examined, 0.25 *M* NaCl would be hypertonic and in some cases extremely so (as much as 250 per cent above normal for the shark sucker).

Although the elasmobranchs do not display the range in variation of isotonic values as do teleosts, it is important to realize that only two-thirds of the osmotic pressure of the blood is due to salts, urea being in large measure responsible for the remaining third. Thus, if 0.5 *M* NaCl were employed as a suspension medium, elasmobranch cells would be at a volume some 20 per cent lower than normal.

Emphasis should again be placed upon the very broad range of isotonic values found within the teleosts. This means that some teleosts have an osmotic pressure of their blood below that of some Amphibia. The indication is that the teleosts, considered as a group, have a more variable regulatory system than the higher groups of vertebrates as regards the solute concentration of the blood.

Summary

1. Isotonic values of the blood of two groups of marine fishes were determined by three physiological methods. These were found to yield approximately the same results.

2. The freezing point depression was used to calculate isosmotic values of NaCl which proved to be generally hypertonic for the associated bloods.

3. The variation of isotonicity in the 14 teleosts examined ranged from 0.26 *M* NaCl for the mackerel to 0.10 *M* NaCl for the shark sucker.

4. Four different species of elasmobranchs were found to have isotonic values of the blood between the limits of 0.33 and 0.35 *M* NaCl. In addition, other commonly used artificial media were evaluated with respect to red cell isotonicity.

5. The extreme variability in the salt concentration of teleost blood, as determined by isotonic studies, emphasizes the need for further study of water regulation in this group of animals.

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